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Graphical Review

The vascular architecture of the pancreatic islets: A homage to August Krogh

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ABSTRACT

The vascular network supporting the islets of Langerhans represents a highly specialised system of arterioles, capillaries and venules. Several features of the islet vasculature (density and fenestration of the capillaries) ensure rapid exchange of nutrients and hormones, which is central to the islets' capacity to control of systemic metabolism via reciprocal changes of insulin and glucagon secretion. Here we discuss how changes in islet blood flow may underlie pulsatile insulin secretion, which becomes impaired in type-2 diabetes. Improved understanding of the architecture and regulation of pancreas/islet blood flow may therefore illuminate the causes underlying this common metabolic disorder. The pioneering work of August Krogh on blood flow, oxygen diffusion and capillary anatomy (that was awarded with the Nobel Prize in 1920) is a cornerstone in these efforts and remains relevant to today's research.

1. Introduction

1.1. Pancreas and the islets of Langerhans: gross anatomy

Type-2 diabetes (T2D) is a common metabolic disorder characterised by impaired insulin secretion. Insulin is secreted by the β-cells of the pancreatic islets, the endocrine part of the pancreas. The insulin secretion defects associated with T2D affect both the kinetics and magnitude of the responses (Rorsman and Ashcroft, 2018). The pancreatic islets are extensively vascularised and this ensures that insulin is efficiently delivered into the circulation and thereby reaches insulin-sensitive organs throughout the body (Rorsman and Ashcroft, 2018). Normally, plasma insulin oscillates with a 12-15 min period. The underlying mechanisms are unclear. Here we will discuss whether islet/pancreas blood flow also plays a role in the initiation and shaping of insulin secretion (Rorsman and Ashcroft, 2018).

Rodents are extensively used in experimental diabetes research. In mice, the pancreas is a diffuse organ situated between the duodenum and the spleen. It is divided in three lobes: the duodenal, the splenic (largest) and the gastric lobes. The duodenal lobe is embedded in the mesentery and the pancreas is intercalated with adipose tissues, making the demarcation of the organ difficult during surgical procedures. In humans, the pancreas is a compact and more well-defined organ divided into three major parts: the head, the body and the tail. The head is situated adjacent to the duodenum, the body below the stomach and the tail in close proximity to the spleen. The pancreas is embedded in a connective tissue capsule, which delineates the lobules of the pancreas. The lobules comprise clusters of tuboloacinar glands that are surrounded by connective tissue, blood vessels, pancreatic ducts and other supportive structures (shown schematically in Fig. 1b). The acini are groups of secretory cells producing digestive enzymes.

The islets of Langerhans consist of five types of endocrine cell (El-Gohary et al., 2012): α-cells (glucagon-producing), β-cell (insulin), δ-cells (somatostatin), ε-cells (ghrelin) and PP-cell (pancreatic polypeptide) (El-Gohary et al., 2012; Dolenšek et al., 2015) (Fig. 1c). The average islet diameter of the islets in humans and mice has been estimated to be 60–130 μm (Rorsman and Ashcroft, 2018). Importantly, islet sizes are not normally distributed and the 10% largest islets account for 50% of the β-cell volume (Rorsman and Ashcroft, 2018; Kilimnik et al., 2012). In general β-cells locate to the core of the islet with α and δ-cells at the periphery (Fig. 1c for mouse model), the distribution is more random in human islets (Cabrera et al., 2006).

1.2. Pancreas main blood supply

The main arterial blood supply to the human pancreas is from the

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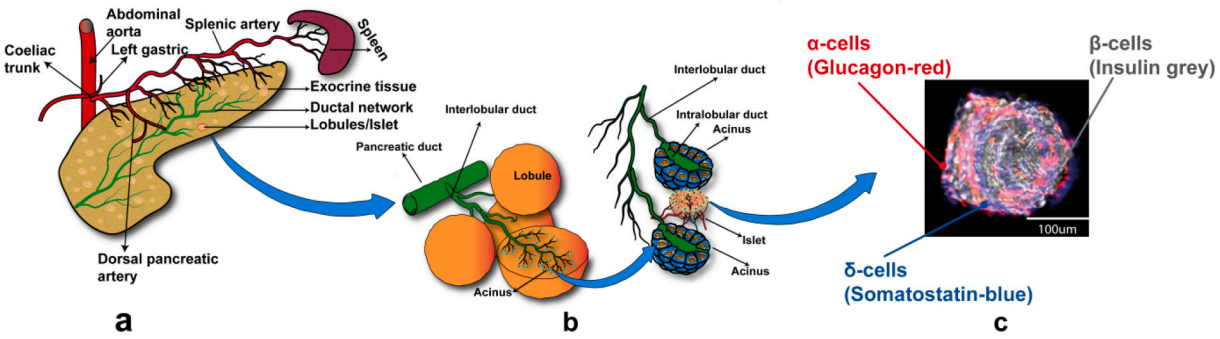

Fig. 1. Macro- and microvasculature of the pancreas. a) Illustration of the main arteries of the human pancreas. b) Lobules and internal structures with associated exocrine ducts. c) Immunostaining and sectioning of a mouse islet. Serial sectioning was used to generate a 3D model of the islet.

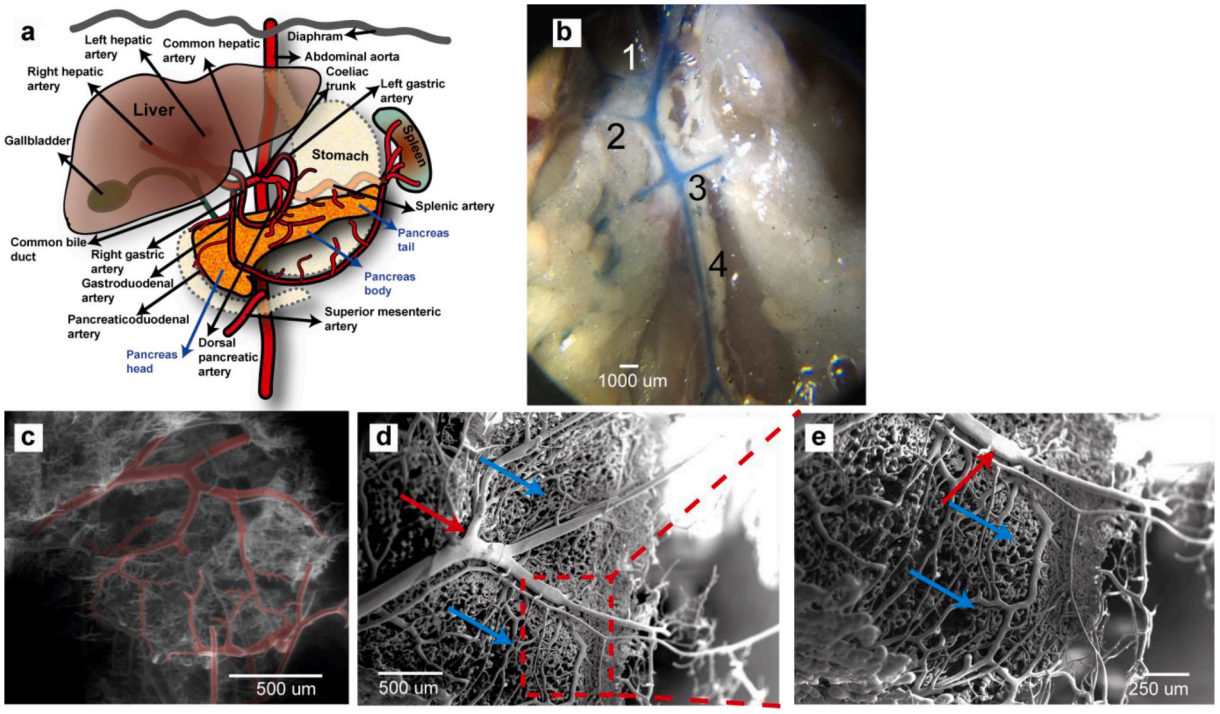

Fig. 2. Vascular casting and SEM images of the microvasculature of the pancreas. a) Schematic of the arterial network of the human pancreas (for clarity, veins and kidneys are not shown). b) Mouse arterial system after resin (methacrylamide) perfusion. Major landmarks in mouse model; the pancreas is folded on the right hand side of the mouse. The numbers are referring to the coeliac trunk (1), superior mesenteric artery (2), right and left kidneys arteries (3) and aorta (4). c) Overall image of capillary network in the pancreas (red = colourised arterioles). The image was taken by SEM from the head of the pancreas. d) Pancreatic vascular network. The red arrow indicates the portal vein with the pancreatic blood vessels seen in the background (blue arrows). e) Detail of vascular network of the pancreas (dashed rectangle in d). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

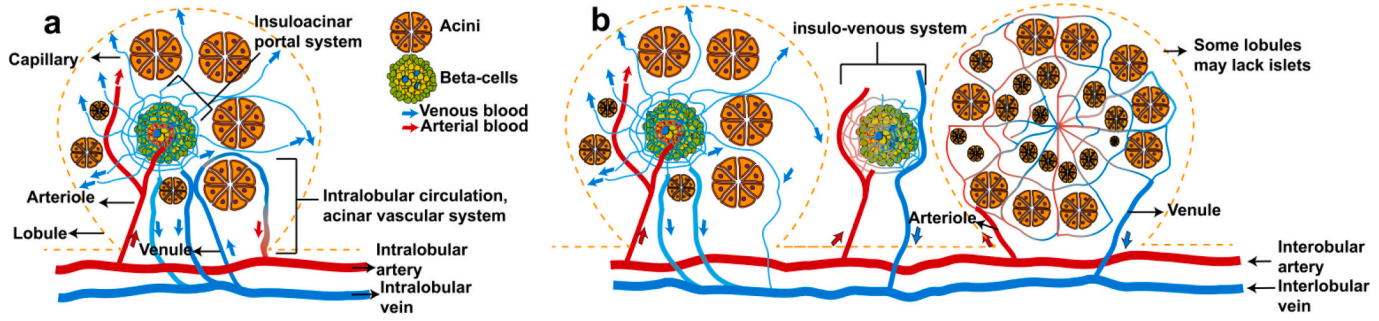

Fig. 3. Islet location and blood supply of pancreatic lobules. a) Representation of a lobule with a central (intralobular) islet and insulo-acinar blood supply. b) Representation of the vascular system in lobules with (left) or without a central islet (right) as well as an interlobular islet where blood flows directly from the islets into the vein ('insulo-venous system'). Note that the illustration is not drawn to scale.

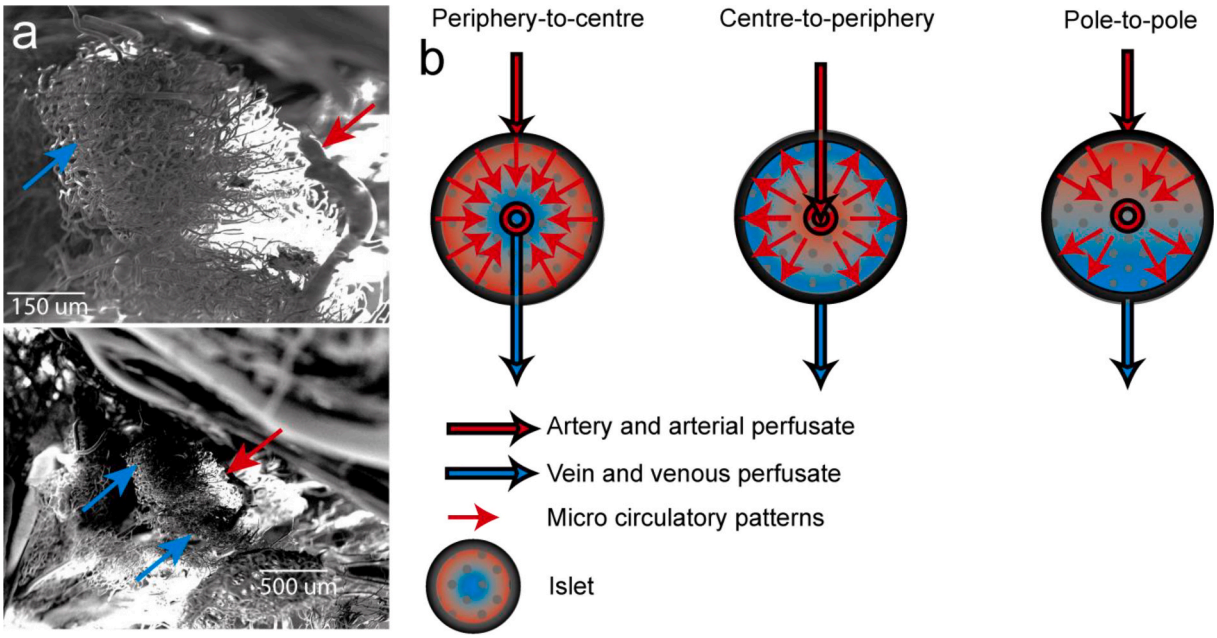

Fig. 4. Blood flow pattern and islets microvasculature. a)An example of an area of dense vascularisation (blue arrows), likely to represent a pancreatic islet. The red arrows indicate an arteriole. The same area of vascularisation is shown at high magnification in the image on top and at lower magnification in the image below.. b) Schematic of different blood flow patterns within islets. Left: Periphery-to-centre; arterial perfusate enter the islet by an arteriole (red arrow) and leaves the islet via a venule (blue) originating in the islet centre. Middle: Centre-to-centre; arteriole enters the islet in the centre and flows towards the periphery before being collected via venules that originate in the periphery. Right: Pole-to-pole; blood flows uniformly from one side of the islet to the other side. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

coeliac trunk, a branch of the descending aorta located below the diaphragm. Blood flow to the pancreas is 1% of the total, which equates (based on a cardiac output of 5 l/min in humans) to ~50 ml/min in humans (Rorsman and Ashcroft, 2018). In mice, pancreatic blood flow is 0.2–0.3 ml/min (Rorsman and Ashcroft, 2018). The head of the pancreas receives most of its blood supply via the gastroduodenal artery with some contribution via the superior mesenteric artery (Kalva et al., 2007; Hagiwara et al., 2016). The body of the pancreas is mainly perfused by the splenic artery and dorsal pancreatic artery (see Fig. 2a). The greater pancreatic artery branches off from the splenic artery and the side branches anastomose with the transverse pancreatic artery (inferior) to supply blood to both head (close to duodenum) and tail (close to spleen) of the pancreas (Kalva et al., 2007). The pancreas is drained via the splenic (body and tail) and pancreaticoduodeal veins (head) into the portal vein (see Fig. 2a). Thus, the liver is the first organ to be exposed to the hormones released by the pancreas. The architecture of the macrovascular network is conserved in humans and rodents (see Fig. 2b, major landmarks in mouse).

1.3. The micro vasculature of the islets of Langerhans

Although the islets only represent 1% of the pancreas, they receive up to 20% of the pancreatic blood flow (Rorsman and Ashcroft, 2018). The understanding of the pancreatic microvasculature in general and the vascular architecture of the islets in particular remains fragmentary. The term 'microvasculature' is used to describe the network of arterioles, venules and capillaries that collectively comprises the microsystem outside and within the islets (Fig. 2c-e and Fig. 3). Consistent with the high blood supply of the islets, vascular density within the islets is 5- to 10-fold that of the surrounding exocrine tissue (Bonner-Weir and Orci, 1982; Konstantinova and Lammert, 2004). Depending on the islet size, each islet receives 1–5 arterioles (El-Gohary et al., 2012; Konstantinova and Lammert, 2004). The capillary network of the islets presents fenestrations, which facilitate the release of the secreted peptide hormones into circulation.

This architecture of the pancreatic microvasculature varies depending on the localisation of the islets and whether the lobule contains an islet or not (Fig. 3a-b) (Bonner-Weir and Orci, 1982; Murakami et al., 1993). The arterioles entering the islets branch out to form the fenestrated capillary network. The efferent (exiting) blood is collected by the venules that then travels to the acini, in effect creating an insulo-acinar portal system (Fig. 3a) (Murakami et al., 1993; Kierszenbaum and Tres, 2012; Liggitt and Dintzis, n.d.; Murakami et al., 1992). Additionally, acini may receive afferent blood directly from the arterial system by an independent acinar vascular system (Fig. 3a) (Murakami et al., 1993; Kierszenbaum and Tres, 2012; Liggitt and Dintzis, n.d.). Studies using vascular casting indicate that some lobules (especially in rodents) lack a central islet (Fig. 3b, right) (Murakami et al., 1993). Whereas the islets reside mainly within the lobules in the human pancreas, many islets locate between the lobules ('interlobular') in mice. In mice (with mainly interlobular islets), both insulo-acinar portal system and insulo-venous system coexist. Interlobular islets drain directly into the interlobular veins with no subsequent interactions with the acini (insulo-venous perfusion; Fig. 3b, middle) (Murakami et al., 1993; Liggitt and Dintzis, n.d.).

1.4. The capillary flow pattern models

The precise conformation of the microvasculature within the islets has only partially been elucidated (Ballian and Brunicardi, 2007). After entering the islets, the arterioles divide into capillaries producing a semi-spherical pod, resembling the structure of a renal glomerulus (Fig. 4a) (El-Gohary et al., 2012; Liggitt and Dintzis, n.d.; Zanone et al., 2005).

Largely based in studies in rodents, three main models for islet perfusion have been proposed (Fig. 4b) (Ballian and Brunicardi, 2007). In the first model ('periphery-to-centre'), blood flows from the periphery to the centre of the islets Fig. 4b, left). In this model, the α-cells in the islet periphery are first exposed to afferent blood and their secreted products may thus affect the function of the β-cells in the islet core

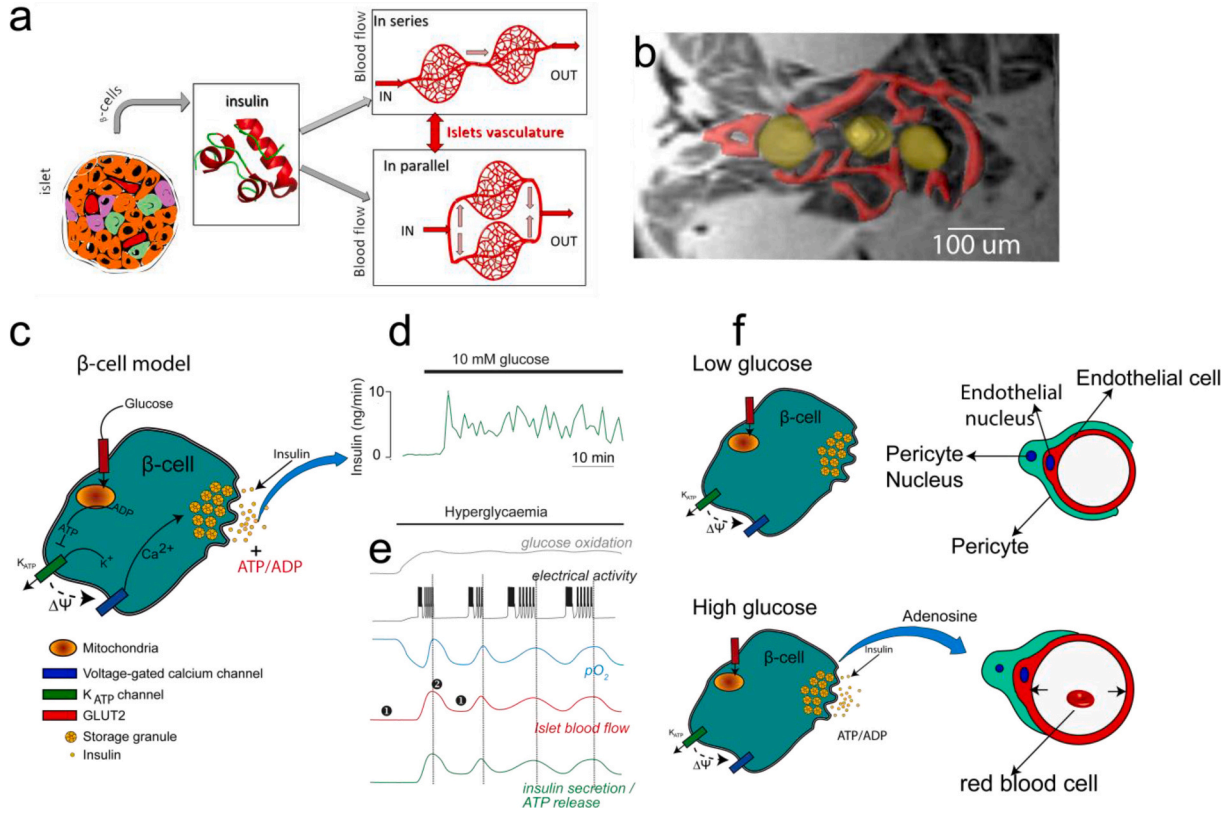

Fig. 5. Schematic representation of islets arrangement and association with oxygen tension and blood flow. a) Schematic depiction of possible vascular distribution between islets. b) Computer reconstruction of islets (yellow), blood vessels (red) and exocrine tissue (grey) by nuclear magnetic resonance imaging. c) Stimulus-secretion coupling in β-cells. See main text for details. d) Pulsatile insulin secretion observed ex vivo in the perfused mouse pancreas. e) Schematic of the possible association between rate of oxidative metabolism (grey), electrical activity (black), insulin secretion (green) and the changes in oxygen tension (pO_2; blue) and blood flow (red). Dashed vertical lines indicate the peak of insulin secretion. f) Proposed relationship between insulin secretion from β-cells, pericyte activity and islet vessel diameter increasing blood flow (indicated by red blood cells). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

before exiting by the venules (Ballian and Brunicardi, 2007). The efferent (insulo-venous drainage) re-enters a secondary capillary network to perfuse the acini (insulo-acinar portal system) as illustrated in Fig. 3a. Whether these blood vessels can re-enter another islet is not known. Obviously, the existence of such inter-islet communication would impact on humoral signalling within the pancreas.

In the second model ('centre-to-periphery'), the blood reaches the core (containing β-cells) first and subsequently flows towards the periphery (Ballian and Brunicardi, 2007; Stagner and Samols, 1992; Brunicardi et al., 1996) (Fig. 4b, middle). This implies that hormones/factors released from the β-cells may modulate the response of the α-cells in the periphery of the islet (Brunicardi et al., 1996). Both insulo-venous drainage and insulo-acinar portal system are compatible with this model (Murakami et al., 1993).

In the third model ('pole-to-pole') the arteriole enters laterally the islet and braches directly into the capillary network (see Fig. 4b, right) (Dolenšek et al., 2015; Ballian and Brunicardi, 2007). This model implies that the blood reaches all cell types simultaneously, permitting a very complex paracrine crosstalk between cell types. Vascular endothelial cells have been proposed to act as internal gates to direct the affluent blood towards different regions of the islets (Konstantinova and Lammert, 2004). These gates not only regulate the rate of perfusion, but may also direct the blood towards regions that contain a particular cell type (Nyman et al., 2010). This model also supports the insulo-acinar and insulo-venous system of blood flow.

Naturally, the different models are not mutually exclusive (Brissova et al., 2005). The centre-to-periphery model predominates in mice (with some islets showing 'pole-to-pole'perfusion) (Brissova et al., 2005). In the human pancreas, some early studies were suggestive of the 'centre-to-periphery'model. In more recent work, the blood vessels entering the islets are lined with α-cells (Bosco et al., 2010) and the other cells are therefore exposed to glucagon and other factors (including glutamate and acetylcholine) released by the α-cells (Rodriguez-Diaz et al., 2011; Cabrera et al., 2008). Whereas the perfusate drains directly in the insulo-venous system for large islets (>60 μm in diameter), the insulo-acinar system seems the principal route of blood drainage for smaller islets (Kilimnik et al., 2012; Murakami et al., 1992; Jansson and Carlsson, 2002).

It is worthy of note that the architecture of the islets is not the same throughout the animal kingdom: whereas rodent islets have a central β-cell core and the non-β-cells cluster to the periphery, the organization is the opposite in some species. For example, in horses and monkeys the β-cells principally locate to the periphery with the non-β-cells in the islet centre (Dolenšek et al., 2015; Murakami et al., 1993). It should also be emphasised that much of the paracrine regulation can occur via diffusion within the islet interstitium without any involvement of the vascular network.

1.5. *Islet vascularization: a homage to August Krogh*

In vivo, plasma insulin oscillates with a period of about 10 min. The underlying mechanism remains an enigma (Rorsman and Ashcroft, 2018). Isolated islets also release insulin with a similar period and this has led to the proposal that the two processes are somehow related. However, it is not at all evident how the secretory activities of all 1 million islets of the human entire pancreas are coordinated. Given that

islet blood flow proceeds 'in parallel' rather than 'in series' (Fig. 5a-b), it seems unlikely that insulin secretion is synchronised by a humoral factor released into the blood vessels. Mechanisms that have been considered include innervation of the pancreas and oscillations in plasma glucose (Rorsman and Ashcroft, 2018). However, oscillations are occasionally observed in the perfused pancreas when innervation has been severed and 'blood' glucose is 'clamped'. Moreover, additions of blockers of nerve activity (like TTX) are ineffective (Sha et al., 2001).[1]

An increase in plasma glucose stimulates insulin secretion. This effect is secondary to an acceleration of oxidative metabolism in the β-cells that increases the intracellular ATP/ADP ratio, closes ATP-regulated K^+ (K_{ATP}) channels leading to membrane depolarization and initiation of oscillatory electrical activity that drives pulsatile insulin secretion (Fig. 5c, d and e). There are strong indications of glycolytic oscillation in β-cells by measurements of metabolic variables including oxygen (Civelek et al., 1997). The acceleration of β-cell oxidative metabolism can be expected to lead to a fall in intra-islet oxygen tension (pO_2). Indeed, such a decrease has been observed in vitro (Kennedy et al., 2002). However, an elegant mechanism operates in vivo to increase islet blood flow to prevent intra-islet pO_2 levels from falling to such low levels that oxidative metabolism (and insulin secretion) is suppressed. Thus, an elevation of circulating glucose leads to increased blood flow in the islet. This regulation involves vascular endothelial cells and the pericytes (Fig. 5e-f) (Ballian and Brunicardi, 2007; Almaca et al., 2018). The pericytes are contractile cells in the blood vessels. They relax (leading to dilation of the blood vessels) at high glucose. There are data indicating that this effect is mediated by activation of adenosine receptors. ATP is co-released with insulin and ectonucleotidase activity in the islets results in its degradation into adenosine (Fig. 5f). The increased blood flow temporarily restores pO_2. The beauty of this model is that blood flow is adjusted to the amounts of insulin secreted.

Oscillatory electrical activity in β-cells (leading to pulsatile insulin secretion) has been attributed to intrinsic biophysical mechanisms and may thus (via reduced adenosine) produce variations in blood flow (Fig. 5) (Rorsman and Ashcroft, 2018). The considerations above raise the interesting possibility that islet blood flow shapes β-cell electrical activity. We acknowledge that the autonomic nervous system is also likely to be involved in vivo but our point is that oscillatory insulin secretion persists in the isolated perfused mouse pancreas (own data and (Matthews et al., 1987)) when innervation has been severed. This suggests that oscillatory insulin secretion is an intrinsic property of the pancreas.

Clearly, the cross-talk between β-cell activity and blood flow shapes represents a new avenue for research to explain how insulin secretion in the entire pancreas is synchronised. Nevertheless, it is clear that the model for the regulation of blood flow in the 'working' islets is very similar to Krogh's observations (made more than a century ago) that capillaries open in skeletal muscle during work and thus increasing blood flow.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

[1] It has been reported in the canine pancreas that TTX abolishes oscillatory insulin secretion. 24. Sha, L., et al., *Amplitude modulation of pulsatile insulin secretion by intrapancreatic ganglion neurons.* Diabetes, 2001. **50**(1): p. 51–5. But this effect is likely explained by the fact that electrical activity in canine β-cells (unlike what is the case in mouse and human β-cells) is strictly dependent on TTX-sensitive voltage-gated Na + channels.

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Space constrains make it impossible to provide experimental procedures used for generation of the data presented in Figs. 2, 4 and 5 but will be supplied on request (massimo.muratore@gu.se).

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